ANALYSIS OF PUTATIVE HYBRIDS OF *HESPEROCYPARIS GLABRA* X *H. PYGMAEA* BY LEAF ESSENTIAL OILS

Robert P. Adams

Biology Department, Baylor University, Box 97388, Waco, TX
76798, USA,   email Robert_Adams@baylor.edu

Frank Callahan
P. O. Box 5531, Central Point, OR 97502

and

Richard Kost
P. O. Box 475, Astoria, OR 97103-0475

ABSTRACT

The leaf volatile oils of three putative, spontaneous hybrids between *Hesperocyparis glabra* and *H. pygmaea* were analyzed and compared to typical oils of the parents as well as twelve F_2s derived from one putative hybrid. The oils of the putative hybrids contained components from both *H. glabra* and *H. pygmaea* (cf. karahanaenone, umbellulone).   Terpenoids present in both parents were present in intermediate or transgressive amounts, confirming the concerned plants to be natural hybrids between *H. glabra* and *H. pygmaea*. The oils from twelve F_2 individuals displayed about equal numbers of intermediate and transgressive components. The use of terpenoids for the analysis of hybridization is discussed. *Phytologia 94(2): 174-192 (August 1, 2012)*

KEY WORDS: *Hesperocyparis* (=*Cupressus*), *H. glabra*, *H. pygmaea*, hybrids, essential oil, terpenes, inheritance, genetics.

There are few studies on the genetics of terpenes in conifers. Hanover (1966) analyzed the genetics of monoterpenes from the oleoresin in clones, F_1 hybrids and S_1 progeny of *Pinus monticola*. He found the inheritance of each terpene (except camphene) to be additive

with some heterotic or epistatic effects. Re-analysis of the Hanover (1966) data for parents and F_1 progeny (Fig. 1) shows that α-pinene is intermediate in 6/17 and transgressive in 11/17 F_1 individuals. β-pinene had 7/17 intermediate and 10/17 transgressive (Fig. 1). δ-3-carene appears to be mostly intermediate (14/17) with only 3/17 being transgressive (Fig. 1), as was the case for limonene (11/17 intermediate, 6/17 transgressive).

Hanover (1971) expanded his study on *P. monticola* and concluded that :
1. Monoterpenes were under strong, predictable genetic control involving one to several loci.
2. One compound, β-pinene, consistently occurred in larger concentrations in the progeny, which may be due to age effects.
3. A strong positive correlation was found between concentrations of δ-3-carene and terpinolene and negative correlations between α-pinene/ β-pinene and myrcene/ δ-3-carene. Otherwise, the compounds appeared to be independently inherited.
4. Negative correlations were found between monoterpene concentrations and progeny height growth rate.

To determine the number of genes controlling terpenes, Irving and Adams (1973) crossed *Hedeoma drummondii* x *H. reverchonii* and analyzed the parents, F_1, and F_2 progeny. They reported the terpenes were controlled by a minimum of 1 to 7 genes.

Squillace (1971) examined inheritance of monoterpenes in oleoresin of *Pinus elliotii* and concluded that β-pinene and myrcene were controlled by two alleles at a single locus, with high amounts being dominant over low. It is interesting to note that this same pattern is evidenced in Fig. 1. Notice, that of the 30 transgressive individuals, 27 are in larger concentrations than the parents (Fig. 1).

Both quantitative and dominance has been reported in the inheritance of terpenes of Douglas fir (von Rudloff, 1984; von Rudloff and Rehfeldt, 1980) and Scots Pine (Pohjola, et al., 1989).
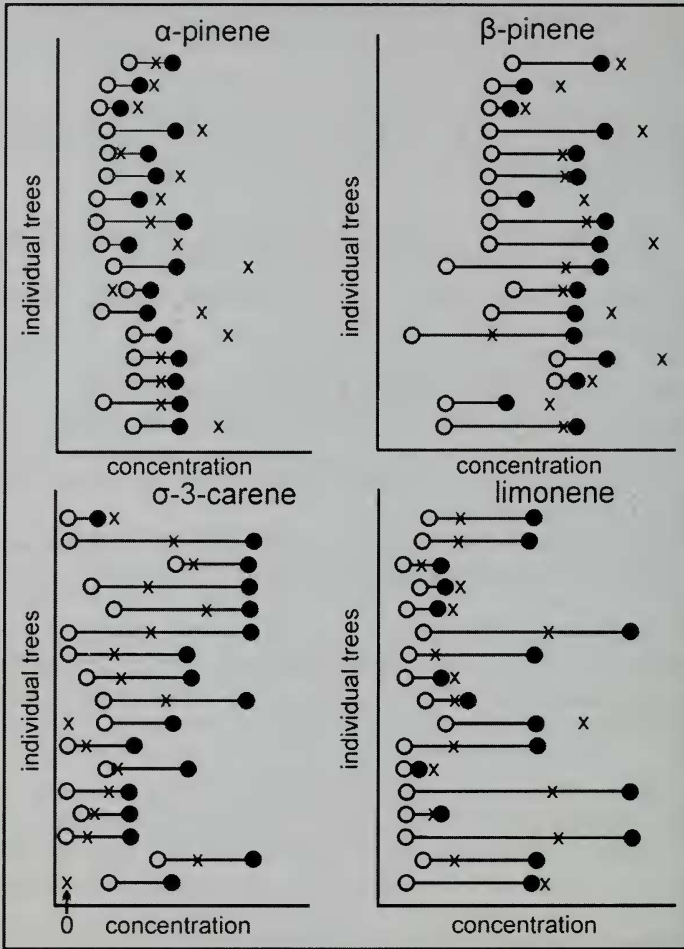
Figure 1. Graphs for four terpenes for 17 F_1 trees. Open and closed circles are the concentrations for parents 1 and 2 in that cross. x is the concentration in the F_1 individual tree. (data from Hanover, 1966).

In the Cupressaceae, there have been very few studies on the inheritance of terpenes. One significant study in *Cupressus* (now *Hesperocyparis*) is that of Lawrence et al. (1975). They examined the

leaf oils of *H. sargentii*, *H. macnabiana* and their putative natural hybrids. In a single population, they analyzed oils from 36 trees and concluded that 12 were *H. sargentii*, 13 were *H. macnabiana* and 10 were intermediate in their oils. PCO analysis of their data (Lawrence et al., 1975, Table 2) found the first and second eigenroots accounted for 27 and 17% of the variance among their samples. These low amounts of variance are likely due to the fact that only two samples of *H. sargentii* and one of *H. macnabiana* were present in the data set. Ordination (Fig. 2) reveals the putative hybrids are quite dispersed between the parental species. From this ordination, it would appear that the putative hybrids (x) likely contained some F_{2s} and backcrossed individuals. However, if transgressive inheritance is involved, that could also explain the wide variation in the putative hybrids.
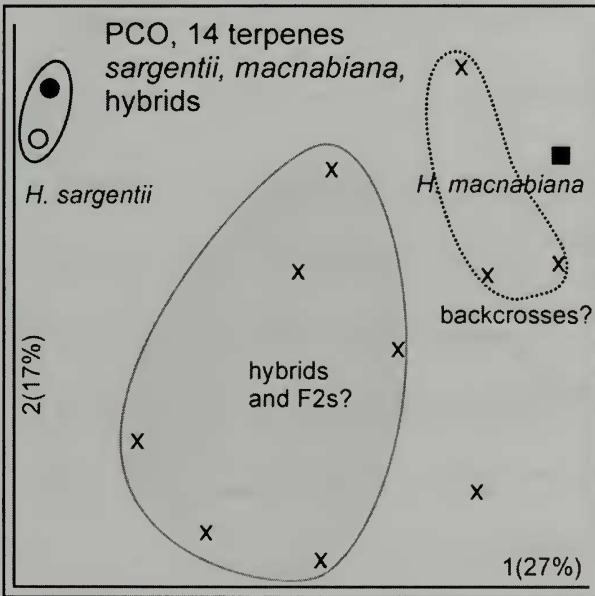

Figure 2. PCO of *H. sargentii, H. macnabiana* and putative hybrids (x). Data from Lawrence et al. (1975, Table 2).

Data from Lawrence et al. (1975, Table 2) was plotted to show the relationship of individual components to the parental species (Fig. 3). Many of the terpenes (7/13) are clearly transgressive. Only 4 of the

13 terpenes are mostly intermediate (camphene, sabinene, myrcene and β-phellandrene). The proportion of transgressive terpenes (7/13) is similar to that found in *P. monticola* (11/17, Fig. 1 above, Hanover,
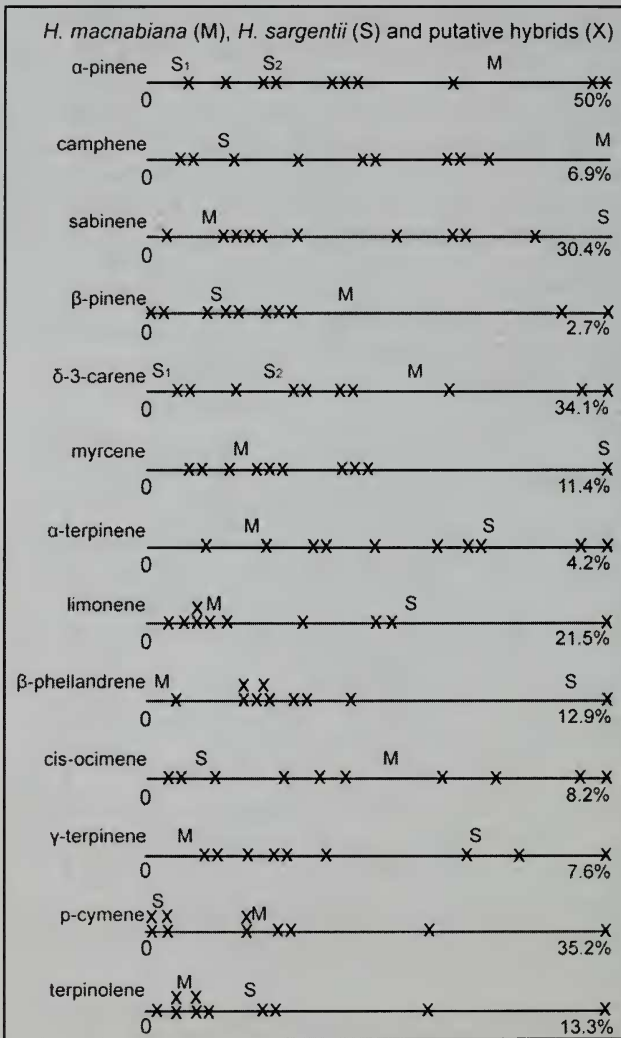

Figure 3. Plot of *sargentii* x *macnabiana* hybrids (data from Lawrence at al. 1975, Table 2). S_1 and S_2 are high/low α-pinene/δ-3-carene popns.

1966). It appears that mixing biosynthetic genes can lead to over-expression of some terpenes.

These 7 transgressive terpenes (α-pinene, β-pinene, δ-3-carene, α-terpinene, limonene, cis-ocimene, p-cymene, terpinolene) plus tricyclene (also transgressive) were removed from the data set and a new PCO performed. This resulted (Fig. 4) in more defined intermediate individuals (presumed hybrids and F_{2s}) and better clustering of the putative backcrosses with *H. macnabiana*. This exercise clearly demonstrates the difficulty of proving hybridization.
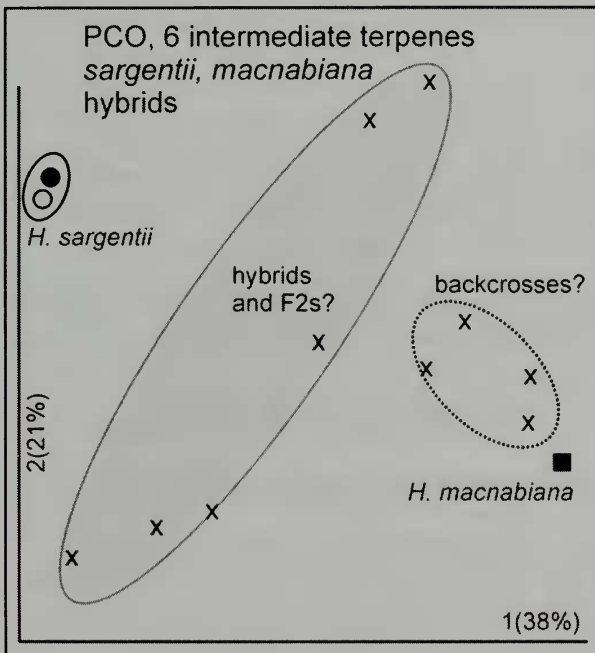

Figure 4. PCO based on 6 mostly intermediate terpenes. Compare with figure 2 using both transgressive and intermediate terpenes.

In 1940, the University of Washington Arboretum accessioned seeds of *H. glabra* and *H. pygmaea* and planted two very glaucous *H. glabra* trees next to a dark green *H. pygmaea* tree near the golf

course in the late 1950s. Richard and Merlin Kost collected seed cones from the *H. pygmaea* (mother tree) in early 1980s. Germination of seeds from these cones produced a few, very unusual, seedlings that were robust with somewhat glaucous foliage (unlike the mother tree, *H. pygmaea*, that was dark green). Two of these seedlings (now large trees) are growing in the Kost Arboretum near Astoria, OR and a third seedling (now a large tree) is growing at the Callahan Arboretum near Central Point, OR. In 2010, leaf samples were taken from these three trees (ca. 42 yrs. old). The original two *H. glabra* trees died in 1974 and the *H. pygmaea* mother tree died in 1997 (pers. comm., Randall Hitchin, Washington Bot. Gard.; unfortunately, these putative parents were not available to study. It appears possible the pollen parents of the seedlings from the *H. pygmaea* tree were from the adjacent *H. glabra* trees. The Callahan tree is very robust and has produced fertile seed for several years. Seedlings and young trees (putative F_2) are very robust and have been cultivated for use as horticultural plants. The nearest cypress is *H. stephensonii* that grows about 3000' from the Callahan putative F_1 tree.

The purpose of this paper is to report on the volatile leaf oils of the putative hybrids, F_2, and parental oils from *H. glabra* and *H. pygmaea*.

MATERIALS AND METHODS

Specimens collected: ***H. glabra***, *Adams 11690-11695*, Dry Beaver Creek, Sedona, 34° 46.131' N, 111° 45.779' W, 1197 m, Yavapai Co., AZ, ***H. pygmaea***, *Adams 11480, 11483* (ex *Bartel 1601a, d*), Albian Ridge, Mendocino Co., CA, *Adams 11484, 11485* (ex *Bartel 1602a, b*), Little River Airport, Mendocino Co., CA, *Adams 11492, 11493* (ex Bartel 1603c, d), Caspar Little Lake, Mendocino Co., CA. putative ***H. glabra x pygmaea*** **hybrids**: *Adams 12444, 12451*, Richard Kost Arboretum, Astoria, OR, *Adams 12452*, Frank Callahan Arboretum, Central Point, OR. **F_2 plants from *Adams 12452***, *Adams 12453-12454, 12458-12475*, Frank Callahan Arboretum, Central Point, OR. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

        Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

        Chemical Analysis - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as percentage of total oil and as mg per g dry foliage weight) compared among the samples by ANOVA and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Gower or Manhattan metric (Gower, 1971; Adams, 1975) were computed among all individuals using character weighting of F-1 (F from ANOVA) for PCO . PCA was performed to examine the patterns of association among individual terpenes in the hybrids (formulation of Gower, 1966 and Veldman, 1967).

RESULTS AND DISCUSSION

        The compositions of the oils of *H. glabra, H. pygmaea*, and putative F_1s are given in Table 1. Twenty three compounds are present in one species but absent in the other species. However, 12 of these compounds (Table 1) are in low concentrations (0.1%) and determining that a compound is absent is questionable, as GCMS sensitivity is so great, even a few molecules can be detected under ideal conditions. There are 11 compounds that are substantially different (conc. in *H. glabra, H. pygmaea*): karahanaenone (0, 14.6%), umbellulone (8.8%, 0), α-cedrene (0.5, 0), β-cedrene (0.3%, 0), cis-muurola-3,5-diene (4.3%, 0), cis-murrola-4(14),5-diene (11.8%, 0), cedrol (1.2%, 0), α-acorenol (3.2%, 0), β-acorenol (0.6%, 0), α-cadinol (0.9%, 0), iso-

pimara-8(14), 15-diene (0, 0.3%), abietadiene (0.9%, 0) and phyllocladanol (0.5%, 0).   Interestingly, only two of these (karahanaenone, iso-pimara-8(14), 15-diene) are present in *H. glabra*, with 9 present in *H. pygmaea*. Two sets of components present in *H. pygmaea* are isomers, and thus may be largely controlled by the same gene (with modifiers): (α-cedrene, β-cedrene), (α-acorenol, β-acorenol), so the differences may overestimated. Karahanaenone and umbellulone are unusual components in species of *Hesperocyparis* and seem good taxonomic markers.   For karahanaenone, the putative F_1s are intermediate (12.1, 1.6, 6.8%). The putative F_1s are also intermediate in their umbellulone concentrations (3.6, 7.2, trace). This supports the theory that C1, K1 and K2 are indeed hybrids between *H. glabra* and *H. pygmaea*. Adams (1982) used leaf terpenoids from *Juniperus* to compare Wells' hybrid distance diagrams, PCA, PCO, and canonical variate analysis. He found that PCO, using character weights of F-1 (F from ANOVA between the putative parents), was the most effective method tested. PCO using 23 terpenoids, shows the putative F_1s cluster near *H. pygmaea*, with K2 being very similar to *H. pygmaea* (Fig. 5). Putative F_1s C1 and K1 are more intermediate in their oils. It is very
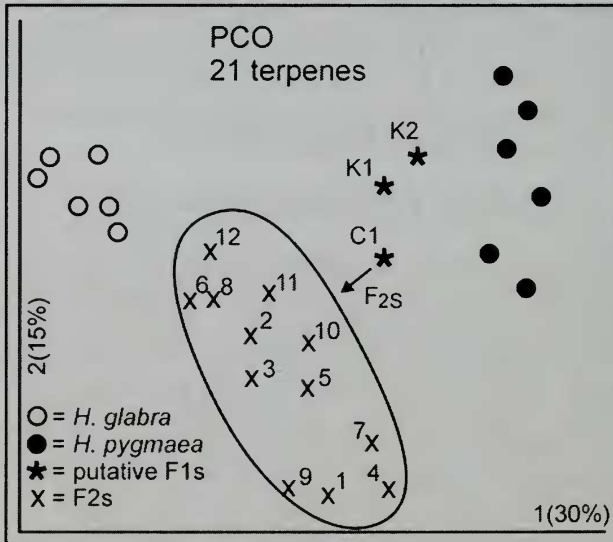

Figure 5. PCO of *H. glabra*, *H. pygmaea*, putative F_1s and F_2s (derived from C1).

important to note that the putative parents of C1, K1, and K2 were not available for study, as they died several years ago and it is unlikely that the oils of the surrogate samples representing *H. glabra* and *H. pygmaea* are exactly like the real parents. The oils of F_2s derived from individual C1 (Fig. 5) have considerable variation. Four F_2s (2, 8, 11, 12) have oils similar to *H. glabra* and four (3, 5, 6, 10) are quite intermediate. Four F_2s (1, 4, 7, 9) are very different. The variation in the F_2s (Fig. 5) is similar to that seen in *H. sargentii - H. macnabiana* (Figs. 2, 3).

Eleven compounds were intermediate in the F_1s and F_2s (Table 1) between the parents: α-thujene, α-pinene, sabinene, α-terpinene, limonene, β-phellandrene, camphor, terpinen-4-ol, α-terpineol, α-terpinyl acetate, and nezukol. These compounds were deemed to be quantatively inherited and are graphed in Fig. 6. Sabinene, limonene, β-phellandrene and camphor were transgressive in the F_2s (Fig. 6). Often one or two individual F_2s contained large, transgressive amounts as in the case of camphor (F_2 #4: 31.8%, F_2 #1: 23%), and limonene (F_2 #5: 20.8%, F_2 #2: 15.5%.

Ten compounds that were present/absent in one putative parent were graphed in Figure 7. For five compounds, most of the F_2s have zero or traces amounts (karahanaenone, α-cedrene, cis-muurola-3,5-diene, cis-muurola-4(14), 15-diene, iso-pimara-8(14), 15-diene). Four compounds have one extremely transgressive individual: camphene hydrate (F_2 #4: 1.5%), umbellulone (F_2 #8: 20.5%), α-acorenol (F_2 #6: 8.1%) and β-acorenol (F_2 #6: 1.6%).

When a compound occurs in a greater concentration (transgressive) than either of the parents, it seems logical to score that individual as being similar to the appropriate parent with high amounts of this component. One way to accomplish this it to truncate the transgressive amount to the level found in one parent. PCO analysis with the 8 transgressive compounds (sabinene, limonene, β-phellandrene, camphor, camphene hydrate, umbellulone, α-acorenol, β-acorenol) truncated at the highest level found in either parent resulted in the removal of more variance in the second eigenroot (17 vs. 15%). Ordination (Fig. 8) shows the F_1s are better resolved from *H. pygmaea* and the F_2s are clustered about the same.
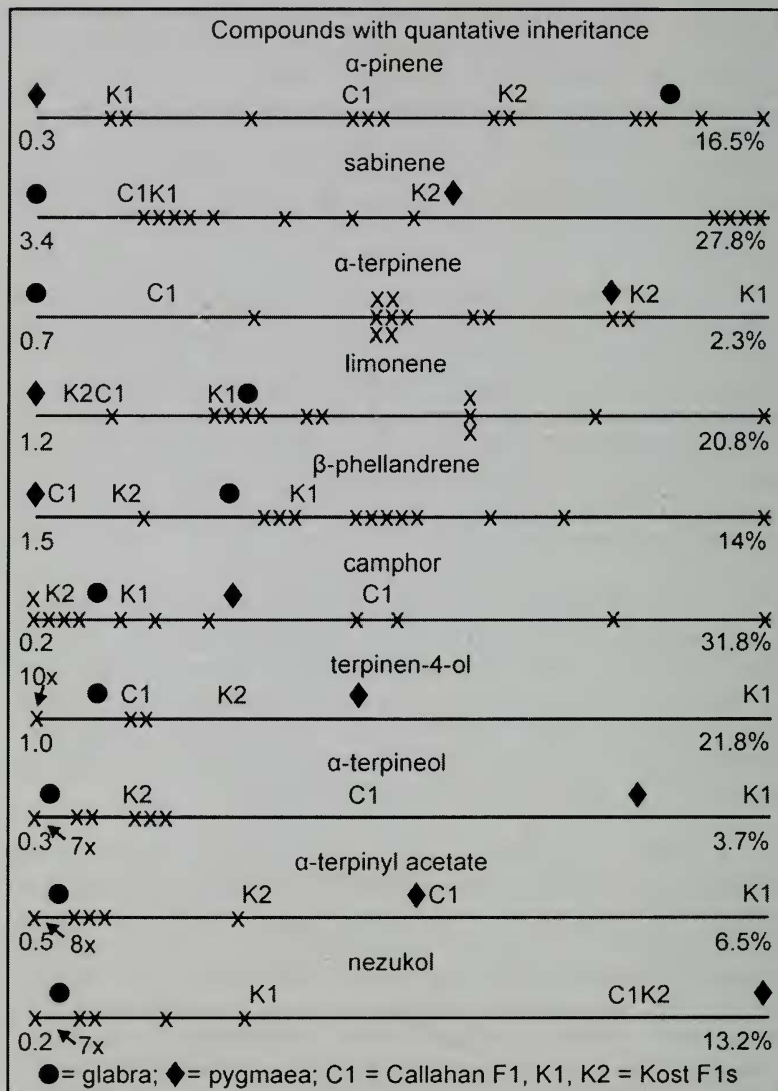
Figure 6. Graph of *H. glabra* and *H. pygmaea*, putative F_1s and F_2s (derived from C1) for terpenes with quantitative inheritance.
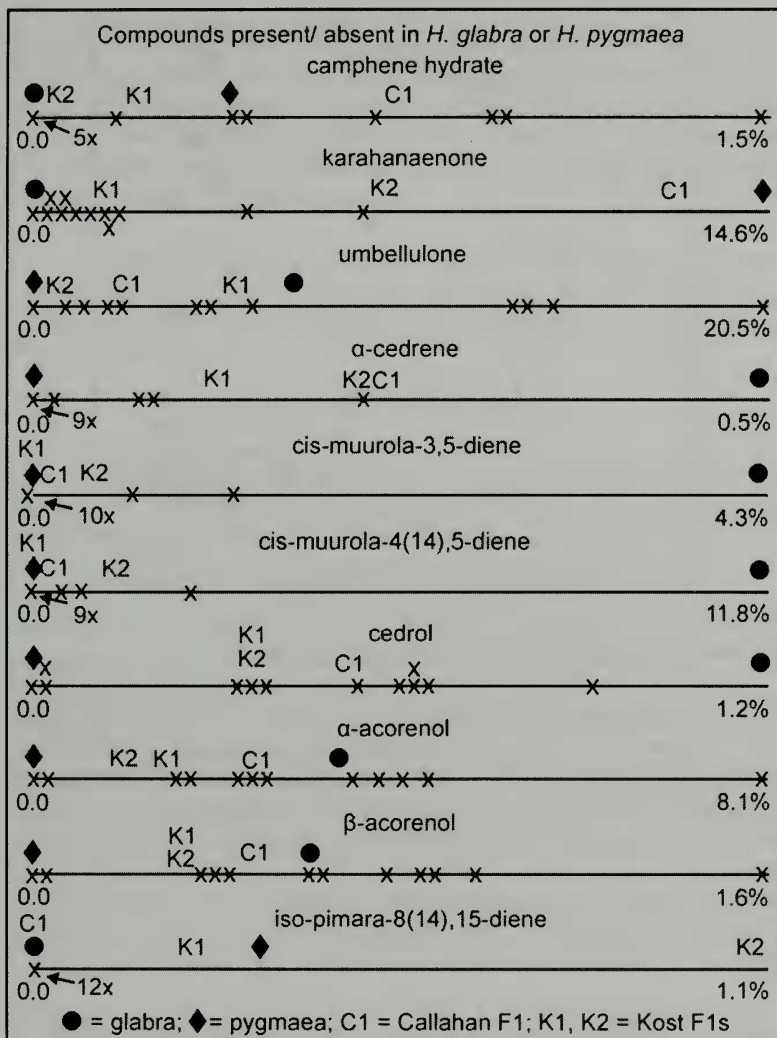
Figure 7. Graph of *H. glabra* and *H. pygmaea*, putative F₁s and F₂s (derived from C1) for terpenes that were present/ absent in one parent.
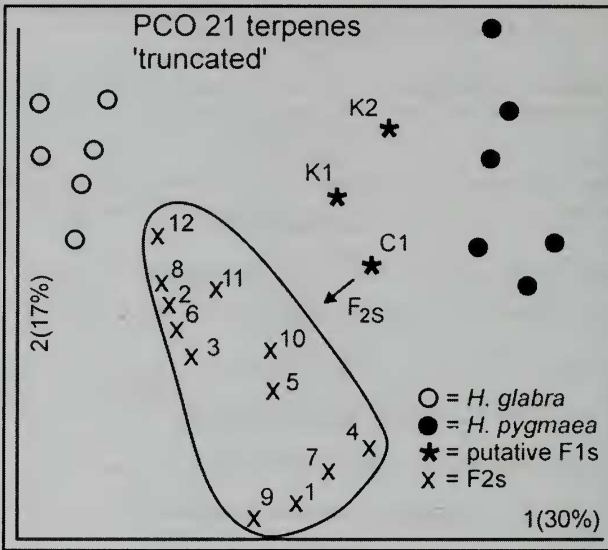
Figure 8. PCO using 21 terpenes with 8 transgressive compounds truncated at the highest level found in either parent.

The nature of inheritance in the present case is similar to that of *H. sargentii* x *H. macnabiana* (Lawrence et al., 1975) and *Pinus monticola* (Hanover, 1966), in that about equal numbers of terpenes are intermediate between parents (versus being transgressive to one of the parents). It is not clear if truncating transgressive characters is appropriate. Data from parents, and their artificial F_1s and F_2s are needed to confirm this methodology. From the present analysis of *H. glabra* x *H. pygmaea*, it may be that *H. sargentii* x *H. macnabiana* plants labeled 'backcrosses' (Fig. 4) are in fact first generation hybrids.

Although it is unfortunate that the parents of the putative hybrids, C1, K1 and K2 died and were not available for analysis, it does appear that these individuals are of hybrid origin. The analysis of F_2s derived from individual C1 demonstrates the large amount of variation one might expect among F_2 individuals, as well as the transgressive nature of many terpenoids.

In a review of transgressive variation (in both plants and animals), Rieseberg et al. (1999) examined 171 studies and found that at least one transgressive trait was reported in 155 (91%) of the studies. Of the 1229 traits studied, 44% were transgressive in the hybrids. Transgressive variation occurred most frequently in intraspecific, inbred, domesticated plants and least frequently in interspecific crosses between outbred, wild animal species.

Clearly, terpene data from parents, and their artificial F_1s (and F_2s) are needed to further elucidate the magnitude of transgressive variation and to evaluate numerical methods for the analysis of putative natural populations.

ACKNOWLEDGEMENTS

LITERATURE CITED

Adams, R. P., 1975. Statistical character weighting and similarity stability. Brittonia 27: 305–316.

Adams, R. P., 1982. A comparison of multivariate methods for the detection of hybridization. Taxon 31: 646-661.

Adams, R. P. 1991. Cedarwood oil - Analysis and properties. pp. 159-173. in: Modern Methods of Plant Analysis, New Series: Oil and Waxes. H.-F. Linskens and J. F. Jackson, eds. Springler- Verlag, Berlin.

Adams, R. P. 2007. Identification of essential oil components by gas chromatography/ mass spectrometry. 4th ed. Allured Publ., Carol Stream, IL.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Hanover, J. W. 1966. Genetics of terpenes: I. Gene control of monoterpene levels in *Pinus monticola* Dougl. Heredity 21: 73-84.

Hanover, J. W. 1971. Genetics of terpenes: II. Genetic variances and interrelationships of monoterpene concentrations in *Pinus monticola*. Heredity 27: 237-245.

Irving, R. and R. P. Adams. 1973. Genetics and biosynthetic relationships of monoterpenes. in: Recent advances Phytochemistry, Vol. 6. pp.187-214. V. C. Runeckles, Ed. Academic Press, N.Y.

Lawrence, L., R. Bartschot, E. Zavarin and J. R. Griffin. 1975. Natural hybridization of *Cupressus sargentii* and *C. macnabiana* and the composition of the derived essential oils. Biochem. Syst. Ecol. 2: 113-119.

Pohjola, J., R. Hiltunen and M. von Schantz. 1989. Variation and inheritance of terpenes in Scots pine. Flavour Fragrance J. 4:121-124.

Rieseberg, L. H., M. A. Archer and R. K. Wayne. 1999. Transgressive segregation, adaptation and speciation. Heredity 83: 363-372.

Squillace, A. E. 1971. Inheritance of monoterpene composition in cortical oleoresin of slash pine. Forest Sci. 17: 381-387.

Vasek, F. C. 1966. The distribution and taxonomy of three western junipers. Brittonia 18: 350-372.

Veldman D. J. 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.

von Rudloff, E. 1984. Inheritance of leaf oil terpene patterns in selfed progeny and some crosses of coastal Douglas-fir. Silvae Genetica 33: 198-203.

von Rudloff, E. and G. E. Rehfeldt. 1980. Chemosystematic studies in the genus *Pseudotsuga*. IV. Inheritance and geographical variation in the leaf oil terpenes of Douglas-fir from the Pacific Northwest. Can. J. Bot. 58: 546-556.

Table 1. Comparisons of the leaf essential oil compositions of putative *H. glabra* x *H. pygmaea* hybrids with the oils of *H. glabra* (glab), and *H. pygmaea* (pyg). Cal F1 = *Adams 12452*, putative *H. glabra* x *pygmaea*, F. Callahan Arb., Ks1 = *Adams 12444*, tree 1, putative *H. glabra* x *pygmaea*, R. Kost Arb., Ks2 = *Adams 12451*, tree 2, putative *H. glabra* x *pygmaea*, R. Kost Arb. Compounds that are present in only one of the parental taxa and in the putative hybrid are in boldface. Compounds that are intermediate in concentration are in italics.

RT	compound	glab	Cal F1	Ks1 F1	Ks2 F1	pyg
846	(E)-2-hexenal	0.3	0.2	0.4	2.7	1.1
908	isobutyl-isobutyrate	-	0.2	0.1	t	-
921	tricyclene	0.1	0.1	t	t	0.1
924	*α-thujene*	*0.6*	*0.2*	*0.2*	*0.7*	*0.6*
932	*α-pinene*	*12.6*	*6.5*	*1.5*	*9.0*	*0.3*
945	α-fenchene	0.1	t	-	0.2	0.1
946	camphene	0.2	0.2	t	0.1	0.3
953	thuja-2,4-diene	t	-	-	-	-
969	*sabinene*	*3.4*	*5.9*	*6.7*	*15.2*	*15.2*
974	β-pinene	0.4	0.3	0.1	1.1	0.3
988	myrcene	1.7	1.8	1.7	2.5	2.6
994	2-octanol	t	-	t	-	-
1002	α-phellandrene	0.2	0.1	0.3	0.2	0.6
1008	δ-3-carene	1.8	0.2	t	5.8	1.3
1014	*α-terpinene*	*0.7*	*0.9*	*2.3*	*2.0*	*1.9*
1020	p-cymene	0.7	0.2	0.3	0.3	0.5
1024	*limonene*	*4.0*	*2.4*	*3.5*	*2.0*	*1.2*
1025	*β-phellandrene*	*4.0*	*1.6*	*5.2*	*2.0*	*1.5*
1026	1,8-cineole	-	-	t	-	0.1
1054	γ-terpinene	1.1	1.6	4.4	3.3	3.1
1065	cis-sabinene hydrate	0.2	0.5	0.9	0.3	0.5
1086	terpinolene	1.0	0.1	2.5	1.8	1.3
1098	trans-sabinene hydrate	0.1	0.6	0.7	0.2	0.3
1099	linalool	0.2	0.6	0.7	0.6	0.7
1100	**n-nonanal**	-	**0.2**	-	**t**	**0.1**
1112	**trans-thujone**	-	**0.1**	-	**t**	**0.1**
1118	cis-p-menth-2-en-1-ol	0.2	0.4	1.4	0.3	0.5
1122	**α-campholenal**	**0.1**	**t**	**t**	**t**	-
1136	trans-p-menth-2-en-1-ol	0.2	t	1.1	0.3	0.3

RT	compound	glab	Cal F1	Ks1 F1	Ks2 F1	pyg
1141	*camphor*	*1.3*	*13.3*	*1.8*	*0.7*	*8.7*
1145	**camphene hydrate**	**-**	**0.7**	**0.2**	**t**	**0.4**
1148	citronellal	t	t	1.4	0.4	t
1154	**karahanaenone**	**-**	**12.1**	**1.6**	**6.8**	**14.6**
1165	borneol	-	t	-	t	t
1167	**umbellulone**	**8.8**	**3.8**	**7.2**	**t**	**-**
1174	*terpinen-4-ol*	*2.7*	*5.4*	*21.8*	*7.8*	*9.5*
1179	p-cymen-8-ol	0.5	0.2	-	-	t
1186	*α-terpineol*	*0.5*	*2.0*	*3.7*	*1.1*	*3.2*
1195	cis-piperitol	-	0.1	0.3	t	t
1195	myrtenal	0.1	-	-	-	-
1205	trans-piperitol	0.3	0.2	0.6	0.1	0.2
1206	verbenone	0.2	-	t	-	-
1223	citronellol	2.4	2.3	2.2	0.3	2.2
1232	thymol, methyl ether	t	-	-	-	-
1239	**carvone**	**t**	**0.1**	**-**	**-**	**-**
1241	**carvacrol, methyl ether**	**-**	**0.1**	**-**	**0.1**	**t**
1249	piperitone	t	0.1	0.2	t	0.1
1287	**bornyl acetate**	**-**	**t**	**0.6**	**0.4**	**t**
1293	2-ethyl-isomenthone	0.2	-	-	-	-
1295	**3-thujanol acetate**	**-**	**0.2**	**1.6**	**-**	**0.1**
1293	2-undecanone	-	-	-	-	t
1299	terpinen-4-yl acetate	0.5	0.1	0.8	0.2	0.1
1339	trans-carvyl acetate	t	-	-	-	-
1346	*α-terpinyl acetate*	*0.6*	*4.3*	*6.5*	*2.6*	*4.2*
1350	citronellyl acetate	-	-	0.2	t	t
1379	geranyl acetate	-	-	0.7	-	-
1403	methyl eugenol	-	-	0.3	-	t
1410	**α-cedrene**	**0.5**	**0.2**	**0.1**	**0.2**	**-**
1419	**β-cedrene**	**0.3**	**0.1**	**t**	**0.1**	**-**
1448	**cis-muurola-3,5-diene**	**4.3**	**t**	**-**	**0.2**	**-**
1452	α-humulene	t	-	-	-	-
1465	**cis-muurola-4(14),5-diene**	**11.8**	**0.1**	**-**	**0.6**	**-**
1465	α-acoradiene	-	0.1	0.1	-	-
1474	10-epi-β-acoradiene	-	t	-	-	-
1479	ar-curcumene	-	t	-	-	-
1480	germacrene D	-	-	t	-	t
1482	citronellol isobutyrate	0.2	-	-	-	-
1501	epi-zonarene	3.9	-	-	-	-

RT	compound	glab	Cal F1	Ks1 F1	Ks2 F1	pyg
1504	cuparene	-	t	-	-	-
1505	β-bisabolene	-	-	-	-	t
1506	(Z)-α-bisabolene	-	0.1	-	-	-
1512	α-alaskene	0.2	0.1	-	-	-
1521	trans-calamenene	2.7	t	-	-	-
1522	δ-cadinene	t	-	-	-	-
1533	10-epi-cubebol	0.4	-	-	-	-
1536	italicene ether	0.9	t	-	-	-
1544	α-calacorene	0.2	-	-	-	-
1550	cis-muurola-5-en-4-β-ol	1.6	t	-	-	-
1559	cis-muurola-5-en-4-α-ol	1.9	t	-	-	-
1559	germacrene B	-	-	-	-	t
1561	(E)-nerolidol	-	0.1	-	-	t
1564	β-calacorene	0.1	-	-	-	-
1582	caryophyllene oxide	t	-	-	-	-
1600	**cedrol**	**1.2**	**0.5**	**0.3**	**0.3**	**-**
1608	humulene epoxide II	0.1	-	-	-	-
1618	epi-cedrol	-	t	t	-	-
1618	1,10-di-epi-cubenol	0.4	-	-	-	-
1627	3-oxobutyl-isomenthone	0.6	-	-	-	-
1632	**α-acorenol**	**3.2**	**2.6**	**1.8**	**1.5**	**-**
1636	**β-acorenol**	**0.6**	**0.5**	**0.3**	**0.3**	**-**
1638	epi-α-cadinol	0.3	-	-	-	-
1638	epi-α-muurolol	0.3	-	-	-	-
1645	cubenol	t	-	-	-	-
1652	**α -cadinol**	**0.9**	**t**	**t**	**-**	**-**
1675	cadalene	0.5	-	-	-	-
1683	epi-α-bisabolol	-	0.1	0.2	-	t
1685	α-bisabolol	-	0.5	-	-	-
1688	cis-14-nor-muurol-5-en-4-one	0.1	-	-	-	-
1722	2Z,6E-franesol	-	-	1.0	-	-
1724	(Z)-nuciferol	-	0.4	-	-	t
1754	β-(Z)-curcumen-12-ol	-	0.4	-	-	t
1887	oplopanonyl acetate	0.1	-	-	-	-
1896	rimuene	-	-	-	0.1	t
1900	pimara-9(11),15-diene	-	t	-	-	1.1
1907	pimara-8(9),15(16)-diene	-	0.1	0.1	0.5	-
1933	isohibaene	t	0.5	0.1	0.5	-
1905	**isopimara-9(11),15-diene**	**-**	**0.4**	**t**	**0.4**	**0.2**

Phytologia (August 2012) 94(2)

RT	compound	glab	Cal F1	Ks1 F1	Ks2 F1	pyg
1948	pimaradiene	-	0.1	-	t	t
1958	**iso-pimara-8(14),15-diene**	**-**	**-**	**0.2**	**1.1**	**0.3**
1960	iso-sandaracopimara-8(14),15	t	-	-	-	t
1966	isophyllocladene	0.4	3.2	0.6	2.9	0.4
1978	manoyl oxide	0.8	2.7	-	3.1	0.5
1987	iso-pimara-7,15-diene	-	-	0.3	-	-
2009	13-epi-manoyl oxide	t	0.6	-	0.4	0.1
2016	**phyllocladene**	**-**	**0.4**	**-**	**0.3**	**0.1**
2055	abietatriene	0.2	0.2	t	0.2	0.1
2087	**abietadiene**	**0.9**	**0.1**	**t**	**t**	**-**
2091	**iso-nezukiol**	**-**	**0.3**	**-**	**1.2**	**0.2**
2105	isoabienol	0.1	-	-	-	t
2132	*nezukol*	*0.6*	*11.7*	*4.4*	*12.1*	*13.2*
2184	**sandaracopimarinal**	**-**	**0.2**	**0.1**	**0.2**	**0.1**
2209	**phyllocladanol**	**0.5**	**t**	**-**	**-**	**-**
2282	sempervirol	0.1	0.5	0.7	0.8	0.3
2314	trans-totarol	0.1	0.1	0.3	0.3	t
2331	trans-ferruginol	t	t	0.1	0.3	t

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.